TREATMENT OF M1 AND M8 HYDROLYSATES WITH HD/TETRYTOL ADAPTED IMMOBILIZED CELL BIOREACTORS

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Under U.S. law and the terms of the Chemical Weapons Convention (CWC), the U.S. Army is required to destroy its stockpile of 30,000 tons of chemical warfare agents by April 2007. While incineration has been the baseline method used for demilitarization of these materials, public and political opposition lead to the evaluation of alternative technologies, including biodegradation. Hot water hydrolysis followed by biodegradation has been shown to be an effective means of disposing of the blister agent sulfur mustard (HD). The ability of this type of immobilized cell bioreactors (ICB's) to deal with a mixture of hydrolyzed HD and Tetrytol (Tetryl and TNT) was evaluated under the Assembled chemical Weapons Assessment (ACWA) program and shown to be quite promising. The work in this presentation deals with a laboratory-scale examination of the ability of ICB's to deal with the hydrolysates of energetics M1 and M8 after grown on HD/Tetrytol. Two sets of ~ 600 ml ICB's in series were inoculated with sewage sludge and biomass from a large-scale ICB and fed a mixture of HD and Tetrytol hydrolysates. After establishment of the cultures, the feed was switched to increasing concentrations of either M1 or M8 hydrolysates as a sole carbon source. The ICB effluents were monitored for COD removal, nitrogen and phosphorus levels, suspended solids and aquatic toxicity. The results of the two systems in respect to their ability to make the changeover from HD/Tetrytol to M1 or M8 and other parameters will be compared and discussed.

INTRODUCTION

One effort currently underway by ACWA is the demonstration of a combined neutralization/ biodegradation process as an alternative to the baseline "incineration" process. A fairly strict application of this process is for siting at the Pueblo Chemical Depot, Pueblo Co. The Pueblo Chemical Depot maintains a stockpile of assembled HD chemical rounds in projectiles and cartridges. The 4.2-inch Mortar and 155mm projectile are examples of these rounds. For a technology to be considered as an alternative to baseline, it must represent a complete solution for the weapon destruction. ACWA has successfully demonstrated the ability of neutralization/biodegradation of the chemical and explosive

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components of these chemical rounds. One process still incomplete is disposition of the rounds propellant charge.

The propellants used in these chemical rounds are the M1 and M8 solid propellants. For this study each of the solid propellants have been hydrolyzed in a 6% NaOH solution. These propellant hydrolysates were fed to two separate laboratory-scale ICB reactors. To closely simulate a likely operation of a proposed full-scale facility, the ICB culture was seeded from biomass removed from the ACWA HD/Tetrytol pilot-scale ICB. The culture was grown-up on HD/Tetrytol hydrolysate feed. After the culture was established the feed was switched to each of the propellant hydrolysates. After the acclimation period and feed concentration run-up, a validation period was started. Samples were taken to measure the biodegradability of the propellant feeds.

METHODS

The M1 and M8 propellants are mixtures of compounds. The propellant materials supplied were removed from 155mm projectiles and shipped to ECBC just prior to the hydrolysate production. The composition of each propellant prior to hydrolysis is listed in Table 1. The propellant hydrolysates have not been completely characterized as of this writing.

TABLE 1. Composition Of M1 And M8 Propellants.

M1 Propellant Composition		**M8 Propellant Composition**	
Compound	% wt/wt	Compound	% wt/wt
Nitrocellulose	85	Nitrocellulose	52.15
Dinitrotoluene	10.0	Nitroglycerine	43.0
Dibutylphthalate	5.0	Diethylphthalate	3.0
Diphenylamine	1.0	Potassium nitrate	1.25
		Ethyl centralite	0.60

The M1 and M8 propellant hydrolysates were prepared by neutralizing in 6% NaOH solution by heating and stirring in laboratory flasks over an 8-hr period. After cooling and coarse filtration the hydrolysates were prepared as bio-feed in 4-liter batches as required. The standard bio-feed formula at full concentration is listed in Table 2.

TABLE 2. Propellant Hydrolysate Feed Formulation.

Compound	**Amount**
Propellant Hydrolysate (M1 or M8)	800 ml
Potassium phosphate di-basic	0.64 gm
Wolin salts	20 ml
Distilled/Deionized Water	To volume (4L)
Neutralize with HCl to pH 7.5	As required for pH=7.5

The laboratory ICB's used for this study are glass cylinders of approximately 1 Liter internal volume. In an ICB the culture grows on an expanded foam media. Spacers mixed with the foam keep the culture from becoming plugged and allow air and aqueous media mixing within the ICB. The actual M8 ICB is shown in Figure 1 below. The expanded foam and spacer packing materials are shown in Figure 2. Under normal growth conditions the working volume of the ICB decreases to approximately 600-ml. Air to supply the culture enters the ICB through a glass fret in the bottom and exits through a tube inserted into a butyl rubber stopper as the top of the ICB. Effluent leaves the ICB through an overflow. For this study two ICB were operated in series for each of the two propellant hydrolysate feeds.

Figure 1. The M8 Propellant ICB.

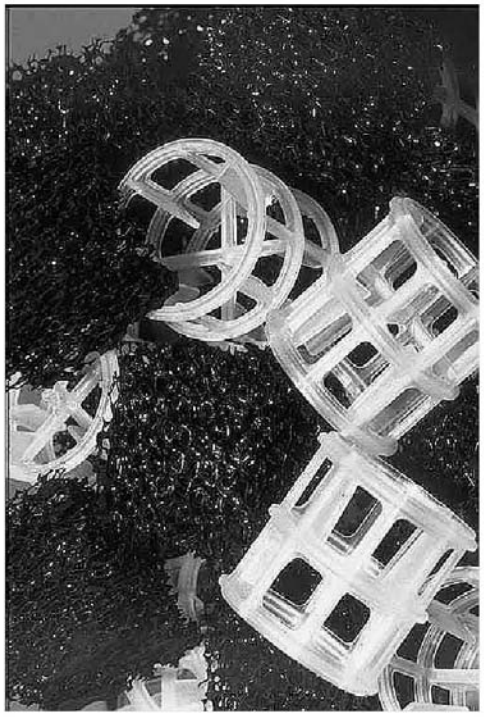

Figure 2. The ICB Expanded Foam And Spacer Media.

The propellant hydrolysate bio-feed was pumped continuously into the ICB at 300 ml/day. Approximately 300 ml/min of air was supplied to each ICB by diaphragm pumps. The media pH was continuously monitored and controlled with acetic acid early in testing to provide additional carbon, and hydrochloric acid during the second half of the 80-day validation period. Process monitoring samples were taken 3-times per week and analyzed for chemical oxygen demand (COD), nitrogen ammonia and phosphate. Samples of the effluent were taken near the end of each feed batch and screened for aquatic toxicity using a MICROTOX Assay.

The MICROTOX (MTX) Bioassay exposes a bioluminescent marine bacterium (*Vibrio fischeri*) to a sample of unknown toxicity and measures the change in light output as the means of determining effects on the organism. A reduction in light output is a direct indication of metabolic inhibition. The assays were performed in glass cuvettes in temperature-controlled wells of a photometer. The assay must have a minimum of four dilutions exhibiting a dose response for optimum accuracy in predicting toxicity. The addition of bacteria was referred to as time zero. Five minutes after time zero the control cuvette was used to calibrate the photometer to 100% light output. The control and treatment cuvettes were returned to the incubator and measured again at 15 minutes. Data was analyzed with the MTX Test Protocol software to determine the EC_{50} (the effective concentration causing a 50% reduction in light out put).

RESULTS AND DISCUSSION

The COD measurement is used as a near real-time measure of the degree of utilization of degradable compounds by the bio-culture. The COD is advantageous in that analysis can be completed in just over two hours. This information is useful in assessing the cultures effectiveness at degrading the propellant feed until more complete analysis is available. At this writing processing monitoring samples

provided the only analytical available. The COD does not indicate degradation or utilization of any one compound of interest, although the COD is mostly associated with carbon compounds and to lesser degree nitrogen containing compounds. The COD of the effluents and the COD removal efficiency of each of the propellant reactors are presented in Figures 3 and 4.

Figure 3 represents the effluent COD results and feeding schedule for the M1 reactor. The culture was inoculated with bacteria from the HD/Tetrytol pilot-scale. The culture was grown-up on HD/Tetrytol feed. On day 150 just prior to changing to propellant feed the COD removal efficiency of the reactors was very good at approximately 90 %. The propellant feed was started on day 153, and is represented by the vertical bars. COD removal efficiency decreased dramatically even though the feed load was greatly decreased. The culture COD removal efficiency improved as the culture adapted even when feed loading was increased. On day 181, additional activated sludge from a local treatment plant was added to supplement the culture. The spike in the effluent COD is a result in adding the carbon rich activated sludge. COD removal efficiency stabilized near day 190. Removal efficiency began dropping even though feed COD decreased as exogenous carbon was gradually removed from the feed and pH control systems.

The 80-day validation period began on day 204. Validation sampling of the effluent began on day 204. Validation sampling results will contain more detailed analysis for the constituents of the ICB effluents and propellant hydrolysate including measures of volatile organic compounds (VOC), metals and mercury, TOC, energetics and nitroglycerine, total dissolved solids, total suspended solids and volatile suspended solids and the Toxic Characteristic Leaching Procedure (TCLP).

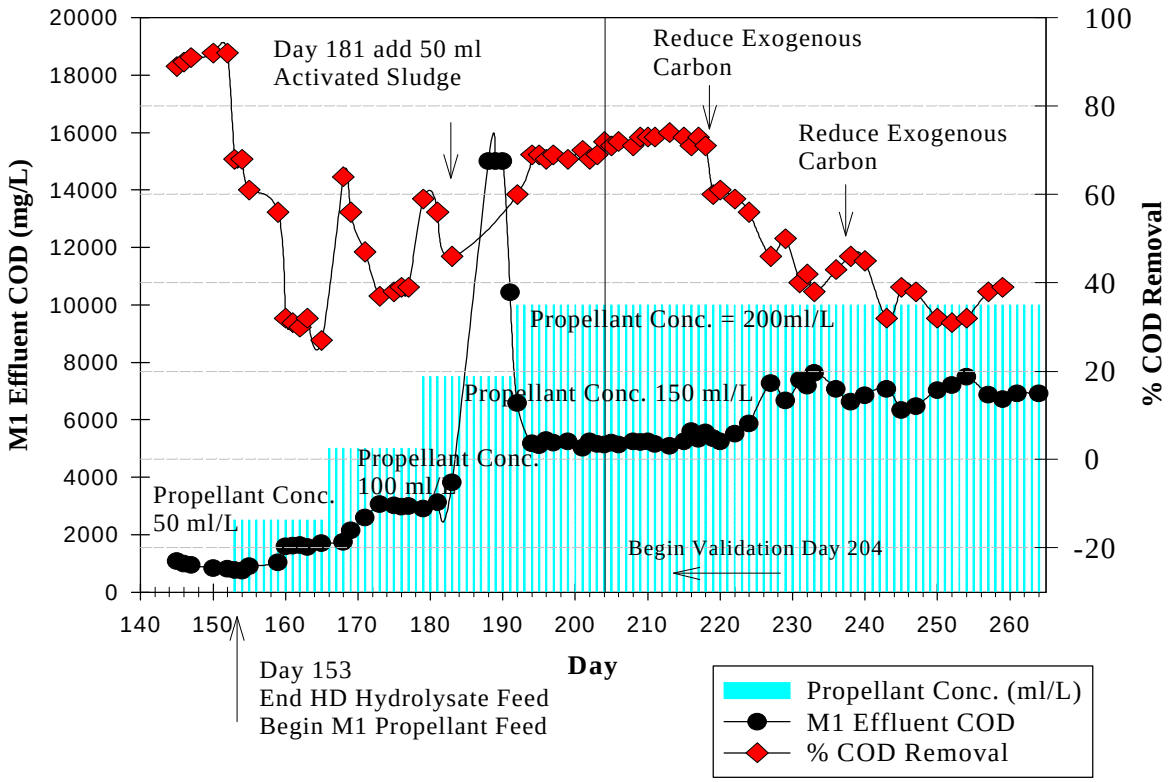

Figure 3. M1 Effluent COD, COD Removal Efficiency and Feed Schedule.

Figure 4 represents the M8 effluent COD, COD removal eficiency and feeding schedule. Like the M1 ICB in figure 3, the M8 ICB was grown on HD/tetrytol feed from an initial innoculumn from the HD/Tetrytol pilot-scale ICB. Change-over to propellant feed and incremental feeding schedule are the same as with the M1 ICB. All changes in pH control, sludge addition, validation start date and exogenous carbon removal are the same. However, the M8 reactor received less exogenous carbon than the M1 reactor due to M8 bio-feed's lower acid requirement for neutralization to pH of 7.5. The M1 feed received 5.4 ml/L acetic acid, while the M8 feed received only 2.5 ml/L acetic acid in the feed.

The effect of change-over to propellant feed, sludge addition and incremental increases in feed loading to each reactor had similar effects on COD removal efficiency. However, the COD removal efficiency of the M1 reactor decreased more dramatically than it did in the M8 reactor.

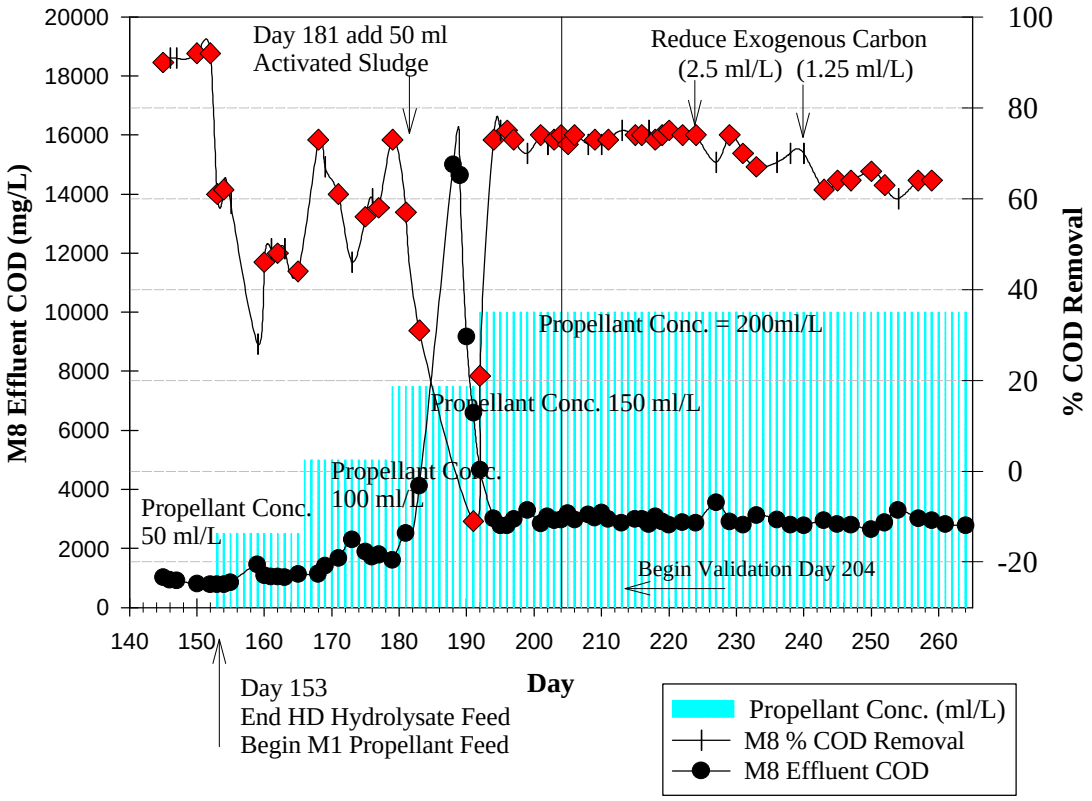

Figure 4. M8 Effluent COD, COD Removal Efficiency and Feed Schedule.

Microtox assays were performed on the propellant feed and ICB effluents during the study. Figure 5 represents the comparative toxicities of the propellant feed at each incremental propellant hydrolysate concentration. The Microtox results for the HD feed are also included for comparison. Microtox results indicated the M1 feed is more toxic than the HD feed at full strength than at the M1 lowest propellant concentration. The M1 at 50 ml/L, its lowest concentration, is more toxic than the M8 feed is at 200 ml/L.

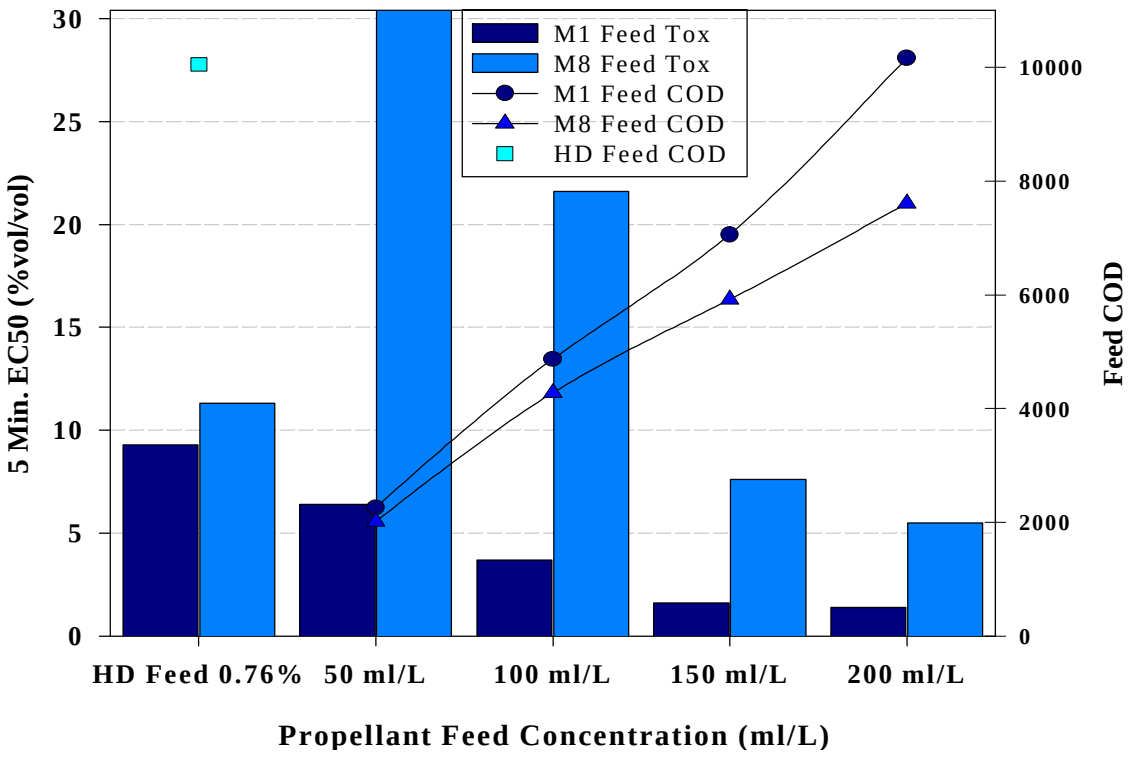

Figure 5. Chart of Microtox And COD Results For ICB Propellant And HD Feeds.

The Microtox Assay results are presented in Figure 6 below. A 5 min. EC50 of greater than 70 is considered non-hazardous. The effluent generated in each of the reactors while being feed the HD hydrolysate is quite low. The toxicity increases immediately after the switch to propellant hydrolysate feed. The M8 reactor recovers shortly after addition of acetic acid to neutralize the feed. The M1 reactor does not do as well with the M1 propellant feed. Once at the target concentration of 200ml/L hydrolysate the M1 reactor effluent becomes quite toxic and hasn't recovered as of day 230, shortly before this writing. The decrease in carbon added through acetic acid seems to have a negative effect on the M8 effluent toxicity, even though COD removal is still quite good (Figure 4.)

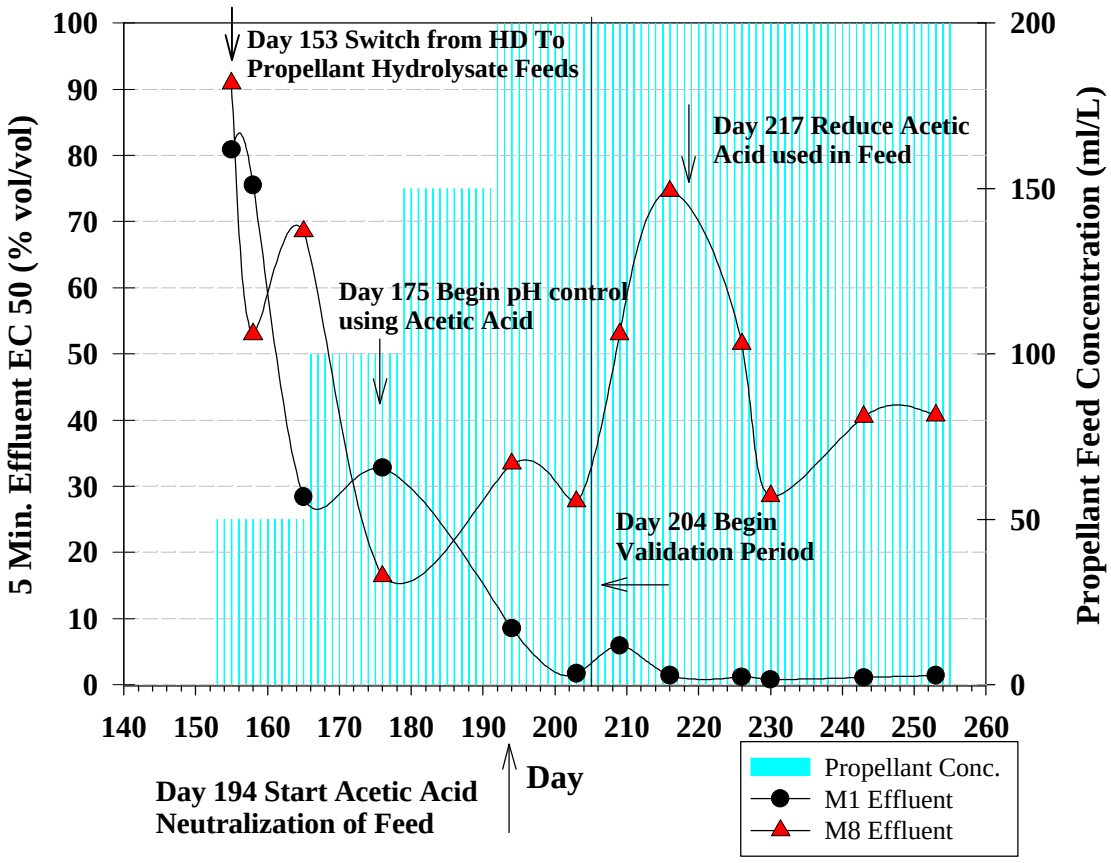

Figure 6. MICROTOX Assay Results For The M1 and M8 ICB Effluents's.

CONCLUSION

As of this writing, planned, in-depth chemical analysis of the propellant hydrolysate and ICB effluents have not been received. We look forward to receiving this data and confirming the performance indicated by our process monitoring data.

The intent of this study was to assess the ICB's ability to degrade the M1 and M8 propellant hydrolysates in to less toxic compounds. The proposed total solution for HD chemical rounds stored at Pueblo Chemical Depot presently under consideration by ACWA limits the flexibility of operating conditions for the reactors. Since the projectile propellant is not considered an agent or Schedule-2 compound under the CWC, it is not subject to a strict destruction deadline. It can therefore be removed from the projectile and stored until after destruction operations for the HD and Schedule-2 compounds are complete. In that scenario, the propellant hydrolysates would be processed separately. The process under consideration also restricts the ICB media carbon to what is already present in the propellant hydrolysates. This study follows that scenario in that it limits the addition of a carbon source that could help in further degrading the nitrogen containing compounds.

From the limited data available from this study, we can see that the ICB culture is able to remove at least 70% of the COD from the feed, indicating that degradation is occurring. Data show that the M8 propellant was degraded to a relatively non-toxic level, as indicated by Microtox assay, while the culture was supplied with an external carbon source. An interesting observation is that since lowering the added carbon the COD removal efficiency has changed little yet the toxicity of the effluent has changed. The

effluent produced since reducing the acetic acid carbon source has become more toxic, confirming an expected relationship between effluent toxicity and carbon availability. The culture still may adapt further to this low carbon condition during the remainder of the study and effluent toxicity may improve.

The M1 reactor effluent has shown consistent increases in toxicity since the switch from HD hydrolysate to the M1 propellant hydrolysate. As of this writing the M1 effluent shows little improvement in toxicity over that of the M1 feed as measured by Microtox Assay. Interestingly the COD removal efficiency of the M1 reactor has declined since removing the external carbon source, a different effect than that observed in the M8 reactor. This effect of decreased carbon and relatively high effluent toxicity still could change over the second half of the study.

Data indicate a need for additional external carbon. A potentially favorable scenario would be to combine the two hydrolysates incorporate them into the HD degradation process, since that process requires the addition of nitrogen compounds to work efficiently. Finally, the addition of a denitrification step to the process could degrade nitrogen containing compounds, releasing previously unavailable carbon for degradation and lessening effluent toxicity.